ISSN(Online): 2319-8753
ISSN (Print): 2347-6710

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 4, Issue 1, January 2015

The Bacterial Flora of Wounds Suffered By Diabetic Patients in Guyana

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ABSTRACT: In a healthy individual the internal tissues are usually free of microorganisms; however, the surface tissue in constant contact with the environment is colonized by a number of indigenous microbiota which is composed mainly of bacteria belonging to about 19 phyla. Some of these bacteria are capable of entering the blood through wounds which provides an optimum environment conducive for colonization. Their abundance and diversity however, is influenced by a number of factors including, the type of wound, its depth, host immune responsiveness and host's demographics. In some cases there are conditions which impair or deteriorate the function of the immune system rendering the host more susceptible to infections which results in rapid colonization of wounds and slow healing rate.
This research, 'the bacterial flora of wounds suffered by diabetic individuals', conducted in 2014 at the Georgetown Public Hospital, aimed to provide substantial evidence to support that a host's demographics can affect the bacterial flora present within that individual's wound. The results yielded from this research support this theory, as it was seen that age has a significant impact on the species diversity of bacteria constituting the wound. While gender had the lesser impact on species diversity, it did affect the individual species of bacteria present within the wound. The results obtained were substantiated after performing statistical analysis (chi square test and t-test).

KEYWORDS:Indigenous microbiota**,** Diabetic**,** Chronic wound**,** Polymicrobial bacterial load**,** Host demographics**,** Pathogenesis**,** Synergistic interactions

I. INTRODUCTION

In a healthy individual the internal tissues are usually free of microorganisms, however, the surface tissues in constant contact with the environment becomes easily colonized by a number of microbes, (Kucharzewski *et al.*, 2006). The organisms which are regularly found on any anatomical site are referred to as the indigenous microbiota.These indigenous microbiota is composed mainly of bacteria belonging to about 19 phyla, most of which are found on the epidermis and the upper portion of hair follicles. The total number of bacteria estimated to be on an average human is 10^{12}. These microbes are usually non-pathogenic, or in some cases beneficial. Such benefits include the prevention of colonization by pathogenic strains of bacteria on the surface of the skin either by competing for nutrients, secreting toxic chemicals against them, or stimulating the skin's immune system to respond to the threat.

While these residential microbes maybe beneficial, some are the causes of many skin diseases and are capable of entering the blood through wounds which provides an optimum environment conducive for the colonization. Their abundance and diversity however, is influenced by a number of factors including, the type of wound, its depth, host immune responsiveness and the host's demographics.Wounds are characterized as being either chronic or acute, the latter is caused by external damage on intact skin, (Bowler *et al.*, 2001), while former is as a result of some form of laceration. Bacteria readily colonize these exposed areas, as they are moist, warm and very nutritious. Upon these

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infections the host's immune system responds to the threat by the production of harmful substances, increase in body temperature, etc., to eliminate these pathogens.
However, there are some hosts which suffer from specific conditions which impairs the function of their immune systems leaving them more susceptible to infections. As a result of this, the colonization of their wounds tends to occur rapidly, causing their wounds to heal at a slower rate than that of other individuals, (Browne *et al*., 2001).The slow healing process and the high populations of possible bacterial pathogens will result in various forms of infections in the host which when left untreated can become detrimental to their health.

II. MATERIALS AND METHOD

Experiments were conducted during the year 2013-14 at Georgetown Public Hospital Corp.Individuals that were selected were suffering from diabetes mellitus.Etiology of the Wound was documented
For the collection of bacterial flora following techniques were used:

- *Technique 1- Swabbing* (Sterile swabs were used to test the level of microbial contamination of the wounds which were then analyzed)
- *Technique 2- Skin Biopsy*(A skin biopsy is the removal of a piece of skin for the purpose of further examination. This technique was implicated when dealing with ulcers and other severe forms of wounds)

Processing

- This step was only necessary upon the use of technique 2 (the skin biopsy method) and included extracting a homogenate from the removed tissue.

Culturing the bacteria obtained

- *Technique 1*(Streaking)
- *Technique 2*Pour plate method (using the homogenate)

Identifying bacterial colonies

- Factors that were used in the identification of the resulting bacterial colonies included the colony morphology, gram staining and the growth of the colonies.

Analyzing data

- The data collected was subjected tostatistical analysis (chi square and t-test).

III. RESULTS AND DISCUSSION

The bacterial flora composition of wounds suffered by diabetic individuals(Table 1)
Of the 22 species of bacteria isolated all were found to be aerobic in nature (with 20 of the species being facultative anaerobes). The lack of obligate anaerobes in the isolated cultures was not because they were absent from the wound but as a result of their presence deeper within the wound, (Kucharzewski*et al.*, 2006). 17 of the species were gram negative, which could be a potential attribute to the infection of these wounds as gram negative species such as *E.coli*, *P.aeruginosa*, *Klebsiella*, *Enterobacter* and *P.mirabilis*, tend to be more pathogenic. On the other hand while gram positive species are less likely to be pathogenic a number of them exhibit some form of pathogenecity such as beta hemolytic *Streptococcus* and *S.aureus*. Bowler and Armstrong stated that *S.aureus*, *P.aeruginosa* and beta hemolytic *Streptococcus* are the most frequent cause of wound infection and delay in wound healing. Similarly, at the ETRS and EWMA consensus meeting in 1998, the presence of *P.aeruginosa* or beta hemolytic *Streptococcus* in a chronic wound indicated the need of antimicrobial therapy.In addition, the presence of a particular species of bacteria may directly promote or demote the growth of another species as demonstrated by Mayrand and McBride with *K.pneumoniae*which enhances the virulence of some *Prevotella* species by providing succinate.

Table 1- The bacterial flora obtained based on gender of the patients

Bacteria	Male (30)	Male %	Female (40)	Female %	Total (70)	Total %
Enterobactersp	1	1.69	1	1.09	2	1.33
Acinetobactersp	2	3.38	2	2.19	4	2.67

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BH Strep Gp G	0	0	1	1.09	1	0.67
C.diversus	1	1.69	3	3.29	4	2.67
C.freundii	1	1.69	1	1.09	2	1.33
E.aerogenes	0	0	1	1.09	1	0.67
E.cloacae	2	3.38	8	8.80	10	6.67
E.coli	7	11.86	12	13.19	19	12.67
Enterococcus sp	0	0	1	1.09	1	0.67
H.alvei	1	1.69	3	3.29	4	2.67
K.pneumonia	1	1.69	2	2.19	3	2.00
M.morganii	3	5.85	6	6.60	9	6
MR S.aureus	6	10.17	4	4.40	10	6.67
NH Strep	0	0	1	1.09	1	0.67
P.aeruginosa	15	25.42	13	14.28	28	18.67
P.alcalifaciens	3	5.85	4	4.40	7	4.67
P.mirabilis	7	11.86	9	9.90	16	10.67
P.rettgeri	1	1.69	3	3.29	4	2.67
P.vulgaris	1	1.69	0	0	1	0.67
Pseudomonas sp	5	8.47	14	15.38	19	12.67
S.aureus	2	3.38	1	1.09	3	2.00
Y.pseudotuberculosis	0	0	1	1.09	1	0.67
Total	59	100	91	100	150	100

Of the 70 patients, 71% suffered from wounds that were polymicrobial (more than one sp of bacteria present) while the remaining 29% suffered from wounds that were monomicrobial. There have been many assumptions about the relationship between the polymicrobial nature of chronic wounds and their inability to heal. Pathogenic bacteria present in ulcerations in the form of mixed microflora are even more virulent and resistant to treatment; (Kucharzewski, *et al.*, 2006). This allows us to hypothesize that synergistic interactions, which developed as a result of the polymicrobiality, contribute significantly to the pathogenesis of infections of chronic wounds. Synergistic bacterial interactions are more important than the exact number of bacteria, because higher variability of species in the bacterial flora is associated with impaired wound healing, (Kucharzewski, *et al.*, 2006).

The effects of age on the species diversity of the bacterial flora obtained(Table 2)
Based on the results it is shown that there is a significant difference in the bacterial diversity among the various age groups.Throughout the various age ranges it is noted that not only does the number of bacterial species change but also the specific type of bacteria being present as well. As seen on graph 1.0, the lowest age ranges show from 4 and 6 species of bacteria respectively, with 3 of the species being mutual within the two age ranges. The third age range has no individuals present. From the fourth to the eighth age range, there is approximately the same number of species present with the 4^{th} and 8^{th} having the most (10). Within these five ranges, it is shown that nine of the species are mutual to at least 2 ranges. In addition, seven species are specific to a single range and are not shared with any of the other five ranges.

The 9^{th} age range shows the presence of 7 species which were all mutual to other ranges. The 10^{th} age range shows the highest number of species (13), with two of the species being specific to this range while the other eleven are mutual to

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ISSN (Print): 2347-6710

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other ranges. The 11th age range showed six species which were all shared with other age groups. The 12th and 13th range shows approximately the same number of species with the latter having the most. The former includes one species that is not mutual to any other group and also having the lowest number species of the thirteen ranges, due to the presence of a single individual within this range.There are four species of bacteria that are unique to a specific age range and are not found in any other. In addition, they are found in the higher age ranges.

The most common species are found in ten of the thirteen ranges and includes *P.aeruginosa and Proteus mirabilis.* Brook reported that the predominant isolates in 58 ulcers were found to be *S. aureus* and *P. aeruginosa*. In addition, Schmidt and colleagues showed that diabetic ulcerations most frequently contained *Enterobacter cloacae* and *P.aeruginosa.*(Kucharzewski, *et al*., 2006); found that *P. aeruginosa* was three times more often isolated from diabetic ulcerations. The next more prevalent species is E.coli which is found in 9 of the thirteen ranges. (Kucharzewski, *et al*., 2006); stated that the next more frequent bacteria found in ulcerations that were diabetic in origin were *Streptococcus pyogenes* and *Escherichia coli.Morganella morganii,* Methicillin resistant *S.aureus* and *Enterobacter cloacae* are found in 7 of the thirteen ranges with Methicillin resistant *S.aureus* and *Enterobacter cloacae* displaying a similar distribution pattern, being absent from the higher age ranges, while *Morganella morganii* is absent from the lower age ranges. *Citrobacter diversus* was found mainly in the higher ranges while *Citrobacter freundii* is found mainly in the middle range. Other bacteria such as *Acinetobacter* and *P.rettgeri* were found bundled together within particular age ranges.It is not quite certain how age affects the type and diversity of microbes on wounds, however, the changes in the skin as a result of age is considered to be an important factor as it influences the microenvironment of the skin, due to the decreased production of sebum, sweat, etc.

Table 2- Bacterial flora present within the various age ranges

Age Range	20-24	25-29	30-34	35-39	40-44	45-49	50-54	55-59	60-64	65-69	70-74	75-79	80-84
# Patients	2	4	0	7	5	6	10	7	9	10	7	1	2
# Bacterial Sp	4	6	0	10	9	9	9	10	7	13	6	3	4
Enterobacter				*						*			
Acinetobacter				*	*	*							
BH Strep										*			
Citrobacterdiversus										*	*		*
Citrobacterfreundii							*		*				
Enterobacteraerogenes							*						
Enterobacter cloacae		*		*	*	*	*	*		*			
E.coli	*	*		*	*	*	*	*		*	*		
Enterococcus								*					
Hafniaalvei				*		*			*	*			
Klebsiellapneumoniae								*		*		*	
Morganellamorganii				*		*	*	*	*		*	*	
MR S.aureus	*	*			*	*	*		*	*			
NH Strep										*			
P.aeruginosa		*		*	*	*	*	*	*	*	*		*
Proteus mirabilis	*	*		*	*	*		*	*	*	*		*
Providenciarettgeri							*	*					

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Proteus vulgaris												*	
Pseudomonas sp	*			*	*	*	*	*	*	*			*
S. aureus		*			*								
Yersinia pseudotuberculosis								*					
Providenciaalcalifaciens				*	*					*	*		

The effects of gender on the species diversity of the bacterial flora obtained

Based on the results obtained, it is shown that there is no significant difference in the bacterial diversity among the various sexes, however when taking into consideration individual species of bacteria, some variations can be seen. There are four species of bacteria that are present only on females and include *BH Streptococcus, NH Streptococcus, E. aerogenes* and *Y. pseudotuberculosis*, while there is one bacterium that is present only on males which is *Proteus vulgaris*.In addition, *P. aeruginosa* is found in higher concentrations on males, followed by *MR S.aureus*.Females however, show higher concentrations of a number of species including *E. coli, E. cloacae, M. morganii* and *P. rettgeri* among others. There is also a few species of bacteria which are found evenly among the sexes, such as *Acinetobacter* and *Enterobacter*, however occurring in small amounts.(Rosenthal, et *al.*, 2011) stated that host's gender shapes skin environment, thereby influencing what is able to colonize men and women. According to (Rosenthal, *et al.*, 2011), women have been shown to have significantly greater bacterial diversity on their hands than men.While there is research supporting variance in the bacterial diversity between the sexes, it is not generalized and is more restricted to specific parts of the skin.

The antibiotic susceptibility of the bacterial flora obtained(Table 3)

Table 3- Antibiotic susceptibility of bacteria

Antibiotic	# resistant	# susceptible
Amikacin	3	4
Ampicillin	4	1
Ampxicillin	6	0
Augmentin	6	2
Cefazolin	2	3
Cefotaxime	1	2
Cefoxitin	1	0
Ceftazidime	0	1
Ceftriaxone	5	9
Cefuroxime	5	8
Cephalothin	7	4
Chloramphenicol	0	1
Ciprofloxacin	5	8
Clindamycin	0	2
Erythromycin	1	0
Gentamicin	4	16
Imipenem	1	2

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Methicillin	1	0
Piperacillin	1	5
Septrin	4	2
Tetracycline	1	2
Tobramycin	1	7
Vancomycin	1	2

Based on the results obtained from the antibiotic susceptibility test, Gentamicin is seen to have a positive effect on a wider range of bacteria, rendering sixteen species of bacteria susceptible.

IV. CONCLUSION

Based on the results obtained from the research carried out, it can be concluded that bacterial diversity of diabetic patients is affected by the host's demographics. It is evident that age has a more significant impact on both the species diversity and types of bacteria present on the host while the effect of gender is not as significant on the species diversity but does show evidence of affecting the type of bacteria present on the host.

ACKNOWLEDGEMENT

The authors would like to acknowledge the contributionsfrom University of Guyana and Georgetown Public Hospital Corp.

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